Association of Zein Classes with Maize Kernel Hardness[1]

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ABSTRACT

The association between maize kernel vitreosity (hardness) and the composition and content of α-, β-, and γ-zeins was investigated. F_2 populations from two pairs of reciprocal flint × floury crosses were fractionated according to density by flotation in sodium nitrate solutions. Zeins were extracted from these fractions and quantified by reversed-phase high-performance liquid chromatography. Statistical analyses indicated that F_2 genotype affected all zein classes. In addition, direction of cross affected α- and 16-kDa γ-zeins, and the individual ears from which F_2 samples were obtained affected α-, β-, and γ-zein amounts and compositions. The strongest apparent correlation was a decrease in amount of 16-kDa γ-zein as kernel density increased in F_2 kernels from the Papago Flour (floury) × Ross Early Flint (flint) cross. α-Zein levels correlated positively, but to a lesser extent, with kernel density in F_2 kernels from the Papago Flour (floury) × Kantz Flint (flint) cross. In the reciprocal F_2 population, however, zein class and kernel density were not significantly correlated. Thus, while zein classes appear to relate to kernel hardness variation in specific normal maize genotypes, we found no consistent relationship between individual or total zeins and kernel density across all populations.

Maize kernel hardness is an important economic trait. Sufficient hardness is necessary to maintain kernel integrity throughout mechanical harvesting, while being handled during marketing (Anderson and Hall 1991), and in storage. Hardness also contributes to grain test weight (Dorsey-Redding et al 1991), a grade-determining factor in the United States. Varieties with high percentages of hard endosperm are desirable for dry milling because they provide high yields of number 1 flaking grits (Hill et al 1991).

Maize kernels consist of varying proportions of vitreous (hard) and floury (soft) endosperm. Varieties of maize with nearly all hard endosperm are called "flint" maize, and those with soft endosperm are called "floury" (Pomeranz et al 1984). "Dent" varieties vary in proportion of hard and soft endosperm. The proportion of hard-to-soft endosperm can also vary because of genotype, position of kernels on the ear, or environment (Watson and Ramstad 1987).

Direct measurement of kernel hardness (e.g., by dissection) has been too slow for evaluation of many samples. A variety of indirect measurements, highly correlated with hardness, have been used for processing, research, and breeding applications. Indirect measurements of hardness include density determinations based on liquid or gas displacement (graduated buret and pycnometer, respectively), specific gravity groupings (floaters test), bulk density (test weight), and grinding resistance (Stenvert instrument), machine vision of whole or cut kernels, and determinations using newer applications, (e.g., near-infrared reflectance and transmittance instrumentation) (Dorsey-Redding et al 1991, Hill et al 1991).

Breeding efforts to enhance physical or compositional end-use characteristics of maize require effective and expedient assessments of phenotypic traits and may be optimized when genetic control of the traits is understood. This has been demonstrated in the development of high-lysine maize varieties with improved nutritional value due to expression of the *opaque-2 (o2)* mutation. These genotypes have softer endosperm and, hence, are more susceptible to ear-rotting pathogens and breakage (Mertz et al 1964, Kneip and Mason 1989). Breeders at the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) and the University of Illinois (Wessel-Beaver and Lambert 1982) introduced modifier genes into *o2* genotypes that restored endosperm hardness while preserving higher lysine content. The resulting maize genotypes were called quality protein maize (QPM) (Vasal et al 1980).

The hardness of maize endosperm relates to its chemical composition. Hard endosperm contains compact, polygonal starch granules, with abundant, directly associated protein matrices. In soft endosperm, starch granules are larger and less aggregated (Robutti et al 1974). Most investigations of maize endosperm composition have focused on zeins, the alcohol-soluble storage proteins that account for 50% or more of total maize endosperm protein. Kernels with the *o2* mutant gene, however, have 50% less zein (Tsai et al 1978).

Zeins can be divided into several structurally distinct classes. α-Zeins, the major class, are proteins with apparent M_r 19 and 22 kDa; β-zeins have M_r 14 kDa; γ-zeins have M_r 16 and 27 kDa; and δ-zeins have a M_r 10 kDa (Larkins et al 1984).

Paiva et al (1991) explored the distribution of γ-zeins in hard and soft portions of kernels from diverse genotypes. They found more total zein in hard endosperm, regardless of genotype. In normal genotypes, amounts of all major zein classes were similarly less in soft as compared to hard endosperm regions. In QPM and *o2* maize, however, there was a more pronounced reduction of α-zein. In eight normal endosperm genotypes, Dombrink-Kurtzman and Bietz (1993) found an average of 3.3 times more α-zeins in hard as compared to soft endosperms. Soft endosperm contained nearly twice as much 27-kDa γ-zein (based on percent) as did hard endosperm. Wallace et al (1990) found that QPM varieties had low levels of α- and β-zeins, compared to normal varieties, and two to four times as much γ-zein as *o2* or normal maize. This suggested a negative relationship between kernel hardness (vitreosity) and high γ-zein levels in non-*o2* kernels but a positive relationship in QPM kernels.

Increased endosperm hardness in maize grown in fertile versus infertile field plots is associated with increased total kernel protein concentration, especially zeins in hard endosperm (Hamilton et al 1951). A statistically significant, but moderately low correlation ($r = 0.33$) between increased total protein levels and kernel hardness has also been observed in modern hybrids (Dorsey-Redding et al 1991). In normal (non-*o2*) maize, however, the relation of

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zein composition to kernel hardness is not yet clear.

We initiated the present study to sample F_2 populations with kernels varying in hardness, seeking quantitative relationships between zein classes and kernel hardness. We used partially inbred lines obtained from a single self-pollinated ear derived from a full-sib family from vitreous (Ross Early Flint and Kantz Flint) and floury (Papago Flour [60-day]) open-pollinated varieties, and their F_1 and F_2 progenies, so a complete range of endosperm hardness was available from related genotypes. We also examined reciprocal crosses of two populations, since reciprocal effects may be important in expression of kernel hardness (Hayes and East 1915, Schwartz 1963, Bergquist and Thompson 1992). Our hypothesis was that α-zeins would correlate positively with increased kernel vitreosity as observed by Dombrink-Kurtzman and Bietz (1993). Knowledge of these relationships would enhance our ability to predict how selection for specific end-value traits, (e.g., hard endosperm or high-protein specialty hybrids) might influence concomitant changes in the associated trait.

MATERIALS AND METHODS

Corn Culture and Sampling

Kantz Flint and Ross Early Flint open-pollinated maize (*Zea mays* L.) cultivars were obtained from the Ohio Agricultural Research and Development Center (OARDC) germ plasm collection. Papago Flour open-pollinated maize was provided by Gary Nabhan (Native Seeds/SEARCH, Tucson, AZ). Individual plants within a full-sib progeny row representing each parental genotype were self-pollinated to produce S_1 progeny. Single-ear derived S_1 plants were grown in the greenhouse during the winter of 1988–89. The Ross Early Flint × Papago Flour hybrid was produced by crossing a single Ross Early Flint S_1 plant (female) with a single S_1 Papago Flour plant (male). For the reciprocal hybrid, the same Ross Early Flint plant was used to pollinate a different plant from the same Papago Flour S_1 family. The Kantz Flint × Papago Flour reciprocal hybrids were produced by crosses between paired single plant crosses between Kantz Flint S_1 plants and Papago Flour S_1 plants. The F_2 and F_2 reciprocal generations were produced by controlled self-pollination of F_1 plants within each of the respective single ear-to-row nursery rows. Four to six ears were harvested for each Kantz Flint × Papago Flour and Ross Flint × Papago and reciprocal cross. The two most uniform, disease-free ears were chosen for sampling. For the Kantz Flint × Papago Flour hybrid, three ears were sampled to obtain representatives of rare segregating classes. Parental varieties, F_1 crosses, and F_2 progenies were grown at The Ohio State University OARDC King Farm, Guerne, OH, in 1989. Parents and progeny were sampled from one location during one season so that comparisons of genotypes were as free as possible of spurious environmental variation. Sample ears were harvested 50 ± 2 days after pollination and dried in a continuous-flow recirculating dryer to 8.0–8.5% kernel moisture. Ears were hand-shelled, and kernels from the middle portion of ears were retained. Samples were backlit with incandescent light to select clean, intact kernels.

Determination of Kernel Hardness

Ten kernels from three ears of each parent and F_1 cross, and at least 40 F_2 kernels from each of two ears from self-pollinated F_1 plants, were used to determine kernel density by flotation at 20°C in sodium nitrate solutions of 1.13–1.31 specific gravity (Bergquist and Thompson 1992). Specific gravities were tested periodically using a hydrometer and adjusted as necessary. Each sample ($8.0 \pm 0.5\%$ moisture) was first put in a beaker with 150 ml of the least concentrated $NaNO_3$ solution. After stirring, the number of floating kernels was recorded. Kernels that did not float were briefly rinsed with deionized water and placed in the next more concentrated $NaNO_3$ solution. Kernels were rinsed with deionized water and dried. By this procedure, F_2 kernels were initially segregated into six specific gravity classes: 1.13–1.15, floury (class 1); 1.16–1.19, semifloury (class 2); 1.20–1.22, intermediate (class 3); 1.23–1.25, semiflint (class 4); 1.26–1.28, flinty (class 5); and 1.29–1.31, extremely flinty (class 6). These class designations corresponded to those of parental samples from the same plots: 70% or more of Papago Flour kernels were floury (class 1); 70% or more of Kantz Flint kernels were flinty (class 5); and 70% or more of the kernels of Ross Early Flint, which has a distinctive phenotype approaching popcorn in hardness and size, were extremely flinty (class 6). For statistical analyses, these six original density levels were reduced to three ranges: 1.19 and below; 1.20–1.25; and 1.26 and above, because not all crosses had kernels in all six levels. The nondestructive flotation technique (Bergquist and Thompson 1992) allowed examination of alcohol-soluble proteins from kernels with known densities.

To test the correlation between this technique and a direct estimate of hardness, we determined milling resistance (hardness) of F_2 Ross Early Flint × Papago Flour kernels. Five kernels were randomly selected from each parental genotype and from five F_2 density classes (samples representing a sixth class were no longer available). Kernel samples ($8.0 \pm 0.5\%$ moisture) were weighed and individually milled for 10 sec in a WIG-L-BUG apparatus (Crescent Dental Mfg. Co., Lyons, IL). Ground meal was then passed through a 210-μm screen. Meal not passing through the screen was weighed. Results of this test were essentially independent of kernel mass (data not shown).

Polyacrylamide Gel Electrophoresis

Ground whole kernels were defatted for 1 hr at 4°C by agitation with 10 vol (v/w) of cold hexane. Hexane was decanted after centrifugation at $200 \times g$ for 10 min. The meal was then air-dried in a laminar-flow air hood. Protein was extracted by adding 3 vol (v/w) of sodium borate buffer, pH 10, containing 1% sodium dodecyl sulfate and 2% β-mercaptoethanol to ~10 mg of defatted meal (Wallace et al 1990). Samples were agitated at room temperature for 2 hr and centrifuged at $16,000 \times g$ for 10 min. Supernatants containing total extracted proteins were divided into two portions. One aliquot was frozen at −20°C for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). To precipitate all zein (Wall et al 1988), 90% ethanol containing 0.5% NaOAc and 2% β-mercaptoethanol was added to the remaining aliquot to a final concentration of 70% ethanol. Samples were agitated for 2 hr at room temperature and centrifuged at $16,000 \times g$ for 10 min in a microfuge (Eppendorf model 5415). Total zein concentration was determined using a Coomassie Blue G dye-binding assay (Read and Northcote 1981) with commercial zein (Sigma Chemical Co., St. Louis, MO) as standard.

SDS-PAGE was performed on 12.5 or 15% gels (Maniatis et al 1982). Gels were stained overnight with 0.06% Coomassie Brilliant Blue G250 (SERVA, New York, NY) in 30% methanol, 10% TCA, and 60% water. Gels were destained with 50% methanol, 40% water, and 10% acetic acid (Wall et al 1988).

Reversed-Phase High-Performance Liquid Chromatography

Individual kernels previously classified according to density were ground to a fine flour in the WIG-L-BUG, and their alcohol-soluble proteins were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) on a Vydac C_{18} column using a modification of an earlier procedure (Paulis and Bietz 1986). For parental and F_1 lines, 5–10 kernels were individually analyzed using a step gradient (Wilson 1991). For F_2 samples, four individual kernels (two per ear) of each of six density classes were analyzed using a linear solvent gradient (Paulis and Bietz 1986). Column effluent was monitored at 210 nm. Data were collected and integrated using computer programs described previously (Paulis and Bietz 1986). The nature of eluting proteins was determined through comparison to chromatograms of samples of known zein classes.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the Statistical Analysis System (SAS) general least squares procedure (PROC GLM). The model included, as sources of variation: (a) mating (Kantz Flint × Papago Flour vs. Ross Early Flint × Papago Flour); (b) cross within mating (floury × flint vs. flint

TABLE I
Mean Squares from the Analysis of Variance for the Alcohol-Soluble Proteins in Maize Kernels from Reciprocal F_2 Populations Derived from Crosses Between Floury and Flint Maize Genotypes

Source	Degree of Freedom	Area					% Area			
		1	2	3	4	Total	1	2	3	4
Mating (M)	1	5,109+[a]	120,134**	3,975	609,679	985,846	24.71*	133.4+	21.88+	3.61
Cross (C) within M (C/M)	2	93	34,034*	2,827	1,055,837	1,414,754	3.08	15.9	13.42	58.29
Ear (E) within C/M (E/C/M)[b]	6	945**	5,025	1,204**	535,779**	465,506**	4.13**	27.3*	5.43**	72.65**
Density (D)	2	325	1,293	689	31,335	36,211	0.75	0.6	2.31*	1.38
M × D	2	211	1,441	1,594**	6,996	13,176	0.50	3.0	3.47**	5.98
C/M × D	4	180	1,659	22	40,892	49,224	0.20	4.1	0.15	4.42
E/C/M × D[c]	7	191	873	252	46,277	46,125	0.38	6.0	0.35	4.69
Residual[d]	53	161	4,871	272	28,509	37,754	0.38	9.1	0.60	6.09

[a] ** = $P < 0.01$; * = $P < 0.05$; + = $P < 0.10$.
[b] Error term for M and C/M.
[c] Error term for D, M × D, and C/M × D.
[d] Error term for E/C/M and E/C/M × D.

TABLE II
Kernel Densities[a] of Flint and Floury Maize Parents and Their Derived F_1 and F_2 Populations

Genotype[b]	Density
Ross Early Flint × Papago Flour	
REF	1.311 ± 0.005 a[c]
PAP	1.148 ± 0.006 d
F_1 (REF × PAP)	1.320 ± 0.000 a
F_1 (PAP × REF)	1.205 ± 0.008 bc
F_2 (REF × PAP)	1.225 ± 0.013 b
F_2 (PAP × REF)	1.197 ± 0.016 c
Kantz Flint × Papago Flour	
KAN	1.229 ± 0.007 ab
PAP	1.151 ± 0.007 d
F_1 (KAN × PAP)	1.255 ± 0.005 a
F_1 (PAP × KAN)	1.234 ± 0.004 ab
F_2 (KAN × PAP)	1.204 ± 0.011 c
F_2 (PAP × KAN)	1.223 ± 0.006 bc

[a] Determined using flotation technique with varying concentrations of sodium nitrate solutions.
[b] REF = Ross Early Flint; PAP = Papago Flour; KAN = Kantz Flint.
[c] Within columns, means followed by the same letter do not differ at the 0.05 level of probability (Fisher's least significant difference test).

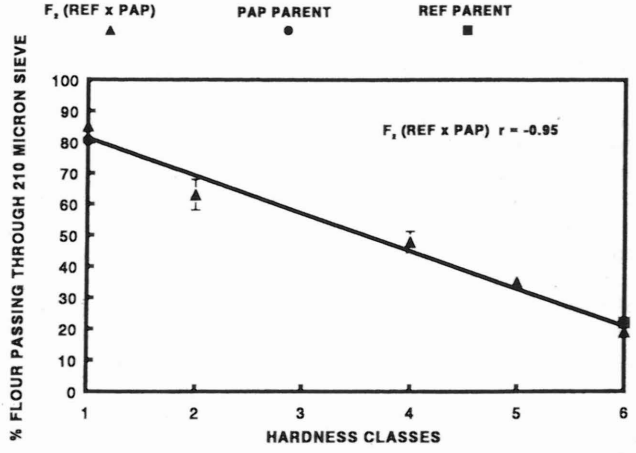

Fig. 1. Association between kernel hardness determined by flotation in sodium nitrate solutions; millability determined using a Crescent WIG-L-BUG apparatus.

× floury); (c) ear within cross and mating; (d) density; (e) the interactions of density with mating, cross within mating, and ear within cross and mating (see Table I). Correlation coefficients were determined within each F_2 population using SAS PROC CORR (SAS 1985).

RESULTS AND DISCUSSION

Of the genotypes we examined, Papago Flour had the least dense kernels as determined by flotation (Table II). Papago Flour had the most meal pass through a 210-μm screen after milling (Fig. 1). Ross Early Flint was the most dense genotype (Table II); only 24% of its meal passed through a 210-μm screen after 10 sec of milling (Fig. 1). F_1 kernels of Kantz Flint × Papago Flour and Ross Early Flint × Papago Flour were as dense as the flint parents (Table II). One reciprocal F_1 cross (Papago Flour × Ross Early Flint) displayed floury endosperm. These results are consistent with a dosage effect on endosperm hardness manifested by the floury-1 *(fl1)* gene (Neuffer et al 1968) and a role of the female parent as the primary determinant of kernel specific gravity (Bergquist and Thompson 1992). The Papago Flour × Kantz Flint kernels were flinty, however. The basis for this result is unknown.

Percent flour yields from 10 sec of milling on a WIG-L-BUG mill were highly correlated ($r = -0.95$) with kernel density (Fig. 1). Visual assessment of vitreosity of previously classified F_2 Kantz Flint × Papago Flour kernels by backlighting techniques also suggested that assigning kernels to floury and flinty classes based on flotation data is consistent. However, vitreousness was not as consistent in assigning kernels to intermediate classes 3–4 (Fig. 2). Other investigators have demonstrated a high degree of correlation between percent of floaters in 1.25 specific gravity solution of sodium nitrate and percent of light transmittance through a single layer of kernels (Hall and Anderson 1991).

Uniformity of kernel protein compositions in S_1 parents was examined using SDS-PAGE in total protein extracts and in zein from 10 kernels from each single ear (data not shown). No differences within genotypes were apparent. Subsequent analyses of additional kernels by RP-HPLC revealed that the five Kantz Flint and 10 Ross Early Flint samples were uniform, but the five Papago Flour kernels showed relatively minor heterogeneity (one or two small peaks present or absent). Papago Flour's SDS-PAGE profile showed more α- than β- or γ-zeins, whereas Kantz Flint showed more γ- than α- and β-zeins (Fig. 3a–c). This quantitative difference was also apparent upon RP- HPLC (Fig. 4). F_1 kernels from Kantz Flint × Papago Flour crosses showed an approximately equal banding profile of γ-, α-, and β-zeins. No consistent relationship was found between zein composition (based on SDS-PAGE pattern) or band intensity and kernel hardness in F_2 kernels. This may be because SDS-PAGE is not a quantitative technique, and zein staining is incomplete (Wilson 1986, 1991).

RP-HPLC analysis (examples of typical data are shown in Fig. 4a–c) was used to quantitatively and qualitatively analyze zein compositions. Individual ears produced differences in both relative and total amounts of the different zeins (Table I). The two Papago Flour × Ross Early Flint populations had greater integrated absorbance areas than did the two Papago Flour × Kantz Flint populations for zein fraction 1 (71.1 vs. 51.1 area units, $P < 0.10$; 3.8 vs. 2.3%, $P < 0.05$). No zein fraction 1 differences were observed for reciprocal crosses or density groups. For zein fraction 2,

Papago Flour and Kantz Flint populations showed greater absorbance areas (266 vs. 177 area units, $P < 0.01$; 12.0 vs. 9.2%, $P < 0.10$). For zein fraction 3, the two Papago Flour and Ross Early Flint populations had an overall higher percent with a negative relationship with density (% area was 4.9, 3.7, and 1.6 at density levels 1, 2, and 3, respectively). The two Papago Flour and Kantz Flint populations had a lower percent of zein 3, which increased with density (% area was 1.4, 1.9, and 2.0, respectively). The mating population by density interaction was significant for % area 3. These results indicate the possibility of distinctly different genetic controls between the flint parents for zein fraction 3 that also display altered maternal and paternal expression. No effects other than the ear differences were found for zein fraction 4.

Correlation analyses (Table III) of individual populations revealed an association between percent of fraction 3 and kernel density (as indicated in the ANOVA model) that was unique to the F_2 Papago Flour × Ross Early Flint population ($r = -0.54$, $P < 0.05$). In the reciprocal cross, the percent of fraction 3 was not correlated with kernel density although the absolute area % was correlated ($r = -0.50$, $P < 0.10$).

The amount of fraction 4 (and of total zein) increased with kernel density in the F_2 Kantz Flint × Papago Flour population ($r = 0.42$, $P < 0.05$) (Table III). However, fractions 1–3 were not significantly correlated to density. This is probably because

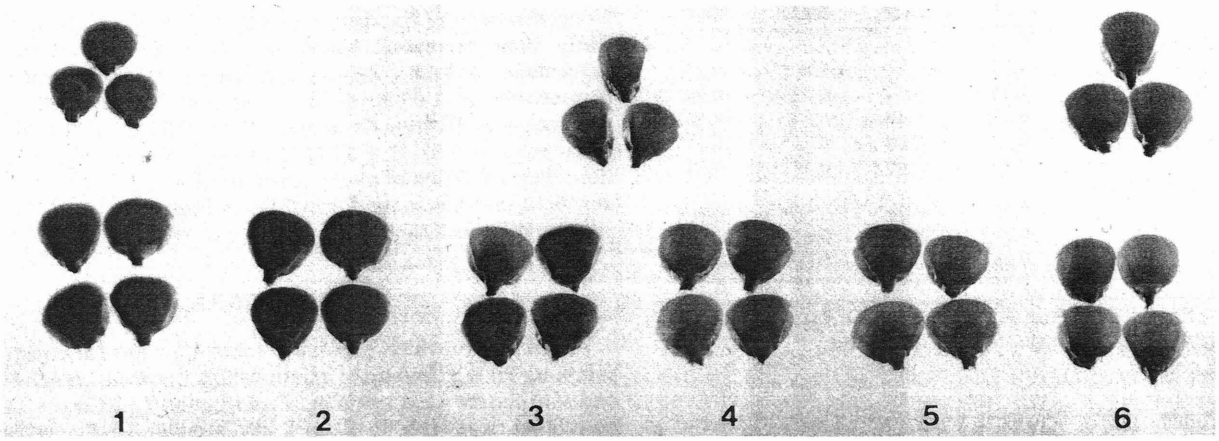

Fig. 2. Visualization of maize kernel vitreosity or opacity by backlighting. Top row: Papago Flour (left); Kantz Flint × Papago Flour (center); Kantz Flint (right). Samples 1–6 in the bottom row are F_2 kernels from the Kantz Flint × Papago Flour population corresponding to density classes 1–6, respectively.

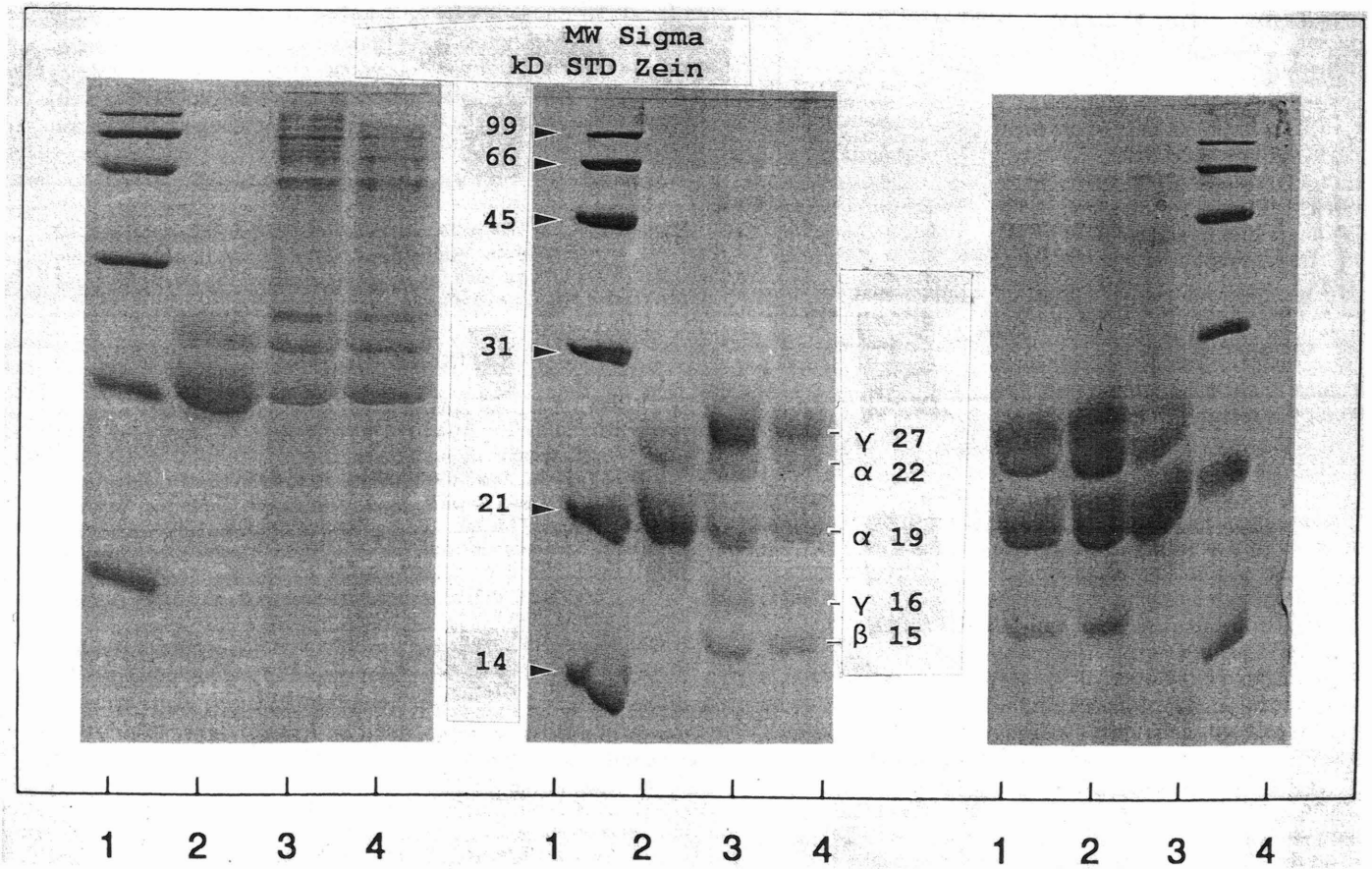

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of total zein fractions from duplicate single kernels of Papago Flour maize (left, lanes 3–4), Kantz Flint maize (center, lanes 3–4), and Ross Early Flint maize (right, lanes 1–2). Sigma zein is shown in lanes 2 (left and center) and 3 (right). Bio-Rad molecular weight standards are in lanes 1 (left and center) and 4 (right).

of the predominance of α-zein in total zein. Thus, as α-zein increased, kernel hardness increased, due to a concomitant increase in amount of vitreous endosperm (Dombrink-Kurtzman and Bietz 1993). However, the expected proportional reduction in γ-zein content (expressed as percent of total zeins), assuming constant total zein concentration did not occur. Also, in the reciprocal Papago Flour × Kantz Flint F_2 population, the amounts of zein of any class, whether expressed as area or percent total area, were not correlated significantly ($P < 0.05$) with density. This indicates that reciprocal effects can occur for this trait and shows that kernel density may increase without any increase in the content of zein classes.

Comparison of the Papago Flour × Kantz Flint and Papago Flour × Ross Early Flint F_2 populations (Table III) indicates that total α-zein in Papago Flour × Ross Early Flint also may be correlated positively with density ($r = 0.46$, $P < 0.05$). Total zein also correlated positively with density, though at a slightly lower level of significance ($r = 0.44$, $P < 0.07$).

Associations between zein amounts and kernel density in F_2 Papago Flour × Ross Early Flint kernels appear unique (Table III). The amount of zein in fractions 1–4 varied significantly. Concentrations of fractions 1 and 3 of F_2 Papago Flour × Ross Early Flint decreased, based on total area and relative area percentage, as kernel density increased ($r = -0.46$ and -0.54, respectively, $P < 0.05$). At the same time, amount and relative percentage of fraction 2 increased ($r = 0.52$, $P < 0.05$), as did total zein ($r = 0.43$, $P < 0.07$). This suggests that, in this population, the correlation of α-zein concentration with increased kernel density is less than in the Kantz Flint × Papago Flour population, yet it is still significant.

TABLE III
Associations of Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Fractions with Maize Kernel Density in F_2 Flint × Floury and Reciprocal Populations

RP-HPLC Fractions	KAN × PAP[a]	PAP × KAN	PAP × REF	REF × PAP
Based on integrated areas				
1	−0.01	−0.30	−0.46*[b]	0.32
2	0.14	0.11	0.52*	0.29
3	0.13	0.19	−0.54*	−0.50+
4	0.42*	−0.08	0.46*	0.17
Total (1–4)	0.46*	−0.06	0.44+	0.34
Based on percentage of total				
1	−0.16	−0.18	−0.53*	0.20
2	0.00	0.13	0.43+	−0.60
3	0.05	0.13	−0.55*	0.02
4	0.03	−0.11	0.38	0.18

[a]KAN = Kantz Flint; REF = Ross Early Flint; PAP = Papago Flour.
[b]* and + = Significant at 0.05 and 0.10 levels, respectively.

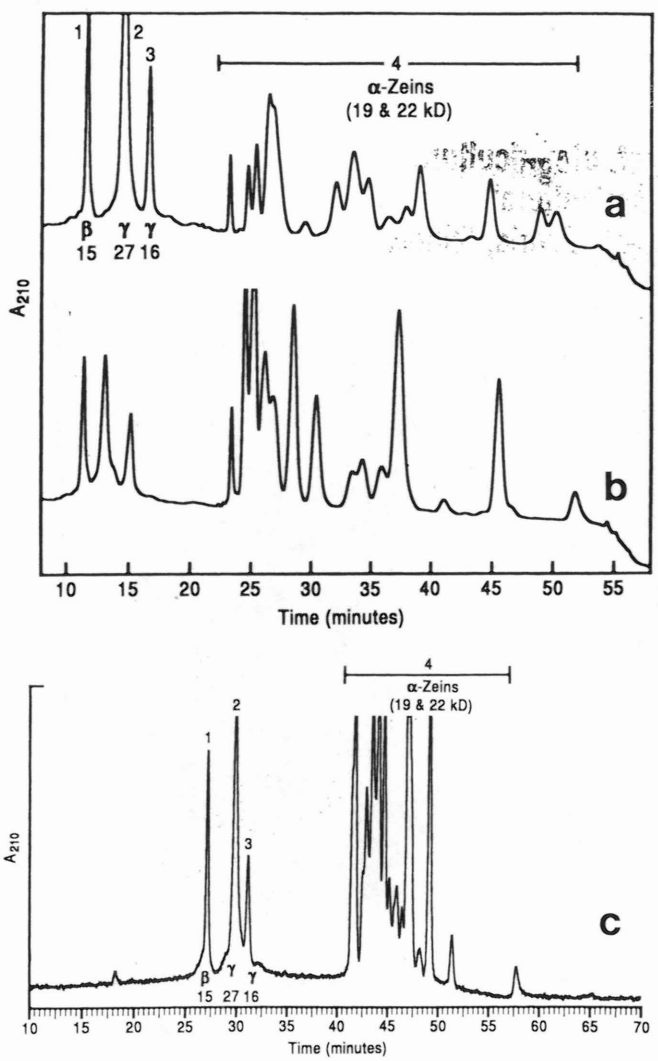

Fig. 4. Reversed phase high-performance liquid chromatograms of total zein fractions from **(a)** Kantz Flint maize, **(b)** Papago Flour maize, and **(c)** Ross Early Flint maize.

CONCLUSIONS

The extent to which genotypic control, reciprocal effects, and sampling (ear) affected the relationships between kernel density and zein classes was surprising. Zein fraction 3 and kernel density correlated negatively in Ross Early Flint derived populations. Kernel density and α-zein correlated positively in only one of several F_2 populations. The association of kernel hardness and density with zein classes is highly genotype-specific, and reciprocal effects exist in some genetic backgrounds. Thus, different relative amounts of zein classes may be associated with wide variation in kernel hardness. It may be possible to modify maize alcohol-soluble protein composition without changing endosperm hardness, but this is genotype-dependent. Breeders may be able to use specific genetic combinations to selectively modify protein composition, hardness, or density without changing the other trait.

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